Theoretical Study of Optical Control of Neurons expressed with Channelrhodopsins

Synopsis

Submitted in the partial fulfilment for the requirement of

Doctor of Philosophy

in

Physics

Submitted by
Nripesh Dixit

Under the supervision of
Prof. Sukhdev Roy

Department of Physics and Computer Science, Faculty of Science
Dayalbagh Educational Institute (Deemed to be University)
Dayalbagh, Agra – 282 005

2024

1. INTRODUCTION

The human brain, one of the most complex systems in the known universe, operates in large-scale functional networks and enables vision, thoughts, memory, learning, hearing, and movement (Krohn *et al.,* 2023; Wang *et al.,* 2020). Understanding the function of the brain is a major challenge to know the biological basis of consciousness, designing brain-computer interfaces, finding causes and cures of various neurological disorders and developing smarter artificial intelligence (Cury *et al.,* 2022; Tang *et al.,* 2023; Yang *et al.,* 2023; Seth & Bayne., 2022; Cho *et al.,* 2022). Although tremendous progress has been made in understanding its architecture, neural connectome, temporal dynamics of brain states, causal connections, and information processing between distinct parts and behavioral activities, much remains unexplored (Shah *et al.,* 2022; Panzeri *et al.,* 2022; Li *et al.,* 2023a). It is therefore essential to develop tools and methods for imaging and to control the activity of neurons in the living brain with high spatiotemporal precision (Lin *et al.,* 2021; 2022; Urai *et al.,* 2022; Wang *et al.,* 2022; Yang *et al.,* 2023). Optogenetics, with its ability to control and monitor genetically modified neurons with the sub-cellular spatial and sub-millisecond temporal resolution, is one of the most promising techniques for studying the brain (Entecheva & Kay, 2021; Kim *et al.,* 2017).

Optogenetics is a powerful neuromodulation and neuroimaging technique that utilizes genetic and optical methods to precisely evoke/suppress or monitor well-defined events in targeted neural populations within living tissue or behaving animals with light (Deisseroth, 2015; Boyden *et al.,* 2005; Emiliani *et al.,* 2022; Entecheva & Kay, 2021; Bansal *et al.,* 2022a). It has revolutionized the way many neuroscience experiments were conducted earlier and thus significantly contributed to our knowledge about the cause and cure of various neurological and psychiatric disorders, information processing and computing in neuron networks, and causal connections between different parts of the brain and behavioral outcomes (Scangos *et al.,* 2023; Li *et al.,* 2022; Gao *et al.,* 2023; Tiwari *et al.,* 2023; Cohen *et al.,* 2023). It has also promising results in cardiology (Entecheva & Kay, 2021; Sung *et al.,* 2022), cell signaling (Repina *et al.,* 2017), the central nervous system (Geng *et al.,* 2023; Parusel *et al.,* 2023), memory modification (Adamczyk *et al.,* 2020), vision restoration (Simunovic *et al.,* 2019; Sahel *et al.,* 2021; Cehajic-Kapetanovic *et al.,* 2022), taste research (Yu *et al.,* 2023), plant science (Christie *et al.,* 2021) and microbiology (Lindner *et al.,* 2022).

In optogenetics, naturally occurring light-sensitive proteins are genetically introduced into the membrane of target neuron (Emiliani *et al.,* 2022; Deisseroth, 2015). After a few days, the protein molecule embeds itself within the cell membrane. On illumination with light, the protein molecule undergoes conformational changes in its structure, leading to a pore-forming across the membrane (Joshi *et al.,* 2020; Kim *et al.,* 2017; Engelhard *et al.,* 2018). This pore acts as an ion-channel, allowing ions to flow across the membrane due to ion-concentration gradient. The optically mediated change in ionic concentration results in a change in neuron membrane potential and thus may trigger or suppress ongoing neural activity (Rost *et al.,* 2017Guru *et al.,* 2015). The performance of optogenetic control of biological systems critically depends on (i) the type of light-sensitive opsin and its expression strategies and (ii) photostimulation methods.

1.1. Light-sensitive Proteins

Light-dependent processes in nature occur in diverse microbial species, plants and animals. Light-sensitive proteins or opsins from microbial algae and bacteria are one of the fundamental components of optogenetics (Emiliani *et al.,* 2022; Zaver *et al.,* 2023; Govorunova *et al.,* 2023). These opsins can be genetically expressed into desired cell populations. In optogenetic control, the type of expressed opsin decides whether it will result in excitation or inhibition. Constant discoveries of new opsins and genetically engineered mutants have resulted in a wide variety of light-gated channels and pumps categorized as excitatory, inhibitory, opsin-pairs for bidirectional control, and step-function opsins or bistable opsins (Bedbrook *et al.,* 2019; Berndt *et al.,* 2015). Characteristics of opsin are defined through its kinetics, conductance, spectral sensitivity, ion-selectivity and binding affinity (Krueger *et al.,* 2019; Xin *et al.,* 2023). Important opsins, their characteristics and their applications are described in Table 1.

1.1.1. Excitatory Opsins

Channelrhodopsins (ChRs) are the most used opsins in optogenetics (Schneider *et al.,* 2015). The main advantage of using these light-gated channels is their fast reversibility, as they remain active only for the duration of light without affecting cell health (Guru *et al.,* 2015). The first experiment showing optogenetic excitation of neurons was performed using ChR2 (Boyden *et al.,* 2005). Illumination of brief blue light pulses on ChR2-expressing neurons results in a train of action potentials with

millisecond temporal resolution. Although ChR2 is still being used for various studies, it has a few drawbacks that include poor light-sensitivity, small photocurrent, blue-activation wavelength, and non-selectivity of ions (Klapoetke *et al.*, 2014; Kojima *et al.*, 2023).

Opsins with red-shifted activation spectra were engineered, as red light can penetrate deeper into the brain tissue in comparison to light at shorter wavelengths due to high scattering (Lin *et al.*, 2013; Mager *et al.*, 2018). Further efforts have been made to enhance the sensitivity of these proteins, which helps in targeting the safe excitation of deeper areas of the brain and provides low-power control.

1.1.2. Inhibitory Opsins

There are two types of opsins resulting in optogenetic inhibition/suppression. Light-driven pumps are the most used inhibitory opsins, which pump Cl^- into the cell or H^+ out of the cell in the presence of light (Engelhard *et al.*, 2018; Marin *et al.*, 2023). Although these pumps are very powerful tools for suppression and hyperpolarization, they require constant illumination of high-intensity light. Furthermore, it is observed that light-driven chloride pumps result in post-illumination excitation due to the constant pumping of chloride ions for a long duration (Wiegert *et al.*, 2017).

Anion-selective ion-channel is an alternative opsin providing suppression of ongoing neural activity in the presence of light (Govorunova *et al.*, 2015). Chloride-conducting channels are efficient tools for making suppression of ongoing neural activity by shunting the membrane voltage near the reversal potential of the opsin channel, i.e., nears the resting membrane potential for Cl^- selective channels. However, these channels may result in excitation rather than suppression due to changes in the chloride concentration gradient (Wiegert *et al.*, 2017). Recently, potassium (K^+) selective ChR named *Hc*KCR1 from *Hyphochytrium catenoids* and WiChR from *Wobblia lunata* has been discovered that exhibits reversal potential near the resting neuron membrane potential (Govorunova *et al.*, 2022; Vierock *et al.*, 2022; Liu *et al.*, 2023). These new opsins have resulted in two-photon holographic inhibition of tens to hundreds of neurons in intact tissue at very low stimulus power 5.74 W and short light pulse, with minimal tissue damage (Vierock *et al.*, 2022).

1.1.3. Opsin Pair for Bidirectional Control

To generate neural codes with the desired spatiotemporal resolution, it is essential to have control over both excitation and inhibition in the same experiment, *i.e.*, bidirectional control. In optogenetics, co-

expression of spectrally orthogonal excitatory and inhibitory opsins allows bidirectional control on illuminating with a blue and orange light pulse. In initial experiments, NpHR and ChR2 were co-expressed into a single neuron population (Zhang *et al.,* 2007). The study shows temporally precise bidirectional control of neural activity and locomotion control in *Drosophila melanogaster* (Zhang *et al.,* 2007; Han & Boyden, 2007).

Capitalizing on the potential of newly discovered excitatory and inhibitory opsins, various new opsin pairs have been reported recently (Gradinaru *et al.,* 2010; Bansal *et al.,* 2020; Vierock *et al.,* 2021). BiPOLES, a Bidirectional Pair of Opsins for Light-induced Excitation and Silencing has been designed using a tandem gene-fusion strategy, which allows potent, light mediated bidirectional control of the same neurons *in vivo* without cross-talk over a wide range of light intensities (Vierock *et al.,* 2021).

Table 1. Comparison of commonly used light-gated ion-channels and -pumps in optogenetics and their characteristics.

Opsin/Opsin-Pair	**Mechanism**	**Peak Wavelength**	**Salient properties**	**Engineering approach**	**Reference(s)**
ChR2	Excitatory Cation Channel	480 nm	Most commonly used excitatory opsin	Native from *Chlamydomonas reinhardtii*	Boyden *et al.,* 2005; Nagel *et al.,* 2003
VChR1	Excitatory Cation Channel	545 nm	Red-shifted opsin	Native from *Volvox carteri.*	Zhang *et al.,* 2008
ChETA	Excitatory Cation Channel	470 nm	Fast kinetics	E123T mutation into natural ChR2	Gunaydin *et al.,* 2010
C1V1	Excitatory Cation Channel	540 nm	Red-shifted opsin	C128 Mutation	Yizhar *et al.,* 2011
Step function opsins (ChR2(C128A) & ChR2(C128S)	Excitatory Cation Channel	470 nm ON 590 nm OFF	Slow channel closure confers bistable kinetics	Single mutations in ChR2.	Berndt *et al.,* 2009
Chronos	Excitatory Cation Channel	500 nm	Fast kinetics	Native from *Stigeoclonium helvectium*	Klapoetke *et al.,* 2014

Chrimson	Excitatory Cation Channel	590 nm	Red-shifted opsin	Native from *Chlamydomonas noctigama*	Klapoetke *et al.*, 2014
NpHR	Inhibitory Chloride Pump	590 nm	First described inhibitory opsin	Native from *Natronomonas pharaonis*	Zhang *et al.*, 2007
eNpHR3.0	Inhibitory Chloride Pump	590 nm	Inhibitory opsin with increased expression level.	NpHR engineered with trafficking sequences from mammalian ion channels	Gradinaru *et al.*, 2010.
GtACR1/2 and RubyACRs	Inhibitory Anion Pump	485, 520, 545 and 590 nm	Naturally occurring chloride ion channel.	Native from *Guillarda theta* and from *Aurantiochytrium limacinum*	Govorunova *et al.*, 2015, 2020
ChrimsonR, f-Chrimson and Vf-Chrimson	Excitatory Cation Channel	594 nm	Ultrafast red-shifted opsins	Derived from *Chlamidomonas Noctigama*	Klapoetke *et al.*, 2014; Mager *et al.*, 2018
CapChR1, CapChR2 and C2LC	Excitatory Anion Channel	470 nm	Calcium permeation with low conductance for sodium	Genetically encoded Ca^{2+} indicators in *Drosophila Melanagaster*	Lahore *et al.*, 2022
HcKCR1, HcKCR2 and WiChR	Inhibitory Anion Channel	520 nm	Fast shuttering and power control	Derived from the stramenopile *Hyphochytrium catenoides* and *Wobblia Lunata*	Vierock *et al.*, 2022; Govorunova *et al.*, 2022
ChRmine, rsChRmine and hsChRmine	Excitatory Cation Pump	~510 nm	Pump like ChRs	Derived from *Tiariva furus and Rhodomonas lens*	Kishi *et al.*, 2022; Marshel *et al.*, 2019; Sineshchekov *et al.*, 2020
Jaws	Inhibitory Chloride Pump	590 nm	Red shifted opsin	Derived from Haloarcula *(Halobacterium) salinarum*	Chuong *et al.*, 2014
ReaChR and bReachES	Excitatory Cation Channel	~600 nm	Red shifted opsin	Developed from ChRs with red spectral shifts VChR1 and C1V1	Lin *et al.*, 2013; Kim *et al.*, 2016
ChroME, ChroME2s and	Excitatory Cation	490 nm	Highly Sensitive and	Soma targeted mutant Chronos,	Sridharan *et al.*, 2022;

ChroME2f	Channel		Fast kinetics	M140E	Mardinly *et al.*, 2018
SOUL	Bistable Control	470 nm ON and 590 nm OFF	Ultrasensitive	Derived by mutation in *Step Function Opsin*	Gong *et al.*, 2020
ChR2-NpHR	Bidirectional Control	470 nm ON and 580 nm OFF	Pump based pair	-	Zhang *et al.*, 2007
ChR2(H134R)-eNpHR3.0	Bidirectional Control	473 nm ON and 589 nm OFF	Pump based pair	-	Gradinaru *et al.*, 2010
GtACR2-Chrimson	Bidirectional Control	490 nm OFF and 600 nm ON	Channel based pair	-	Vierock *et al.*, 2021

1.2. Light Illumination Methods

All the light-sensitive proteins used in optogenetics have their peak activation within the visible spectral range. As discussed earlier, it was initially developed for neurons in the brain. However, it is now being applied to other systems that include cardiac applications and vision restoration. Furthermore, it includes various levels of targeting preparations such as cultured cells, tissue slices, transparent organisms, or the cortical surface of the brain. Additionally, single- and multi-target excitation is preferred for controlling complex activity patterns. Thus, a wide range of optical illumination strategies and tools have been developed for Optogenetics (Emiliani *et al.*, 2022; Li *et al.* 2023b).

1.2.1. Wide-field Illumination

Wide-field illumination or 1-photon excitation approaches stand out as an important component of light-targeting photostimulation strategies. Patterned illumination with wide-field illumination using digital mirror devices have various advantages over two-photon patterned illumination as the required hardware is relatively simple to use and cost-effective, and one can readily co-stimulate larger numbers of neurons with minimal heating (Adesnik *et al.*, 2021). However, it is restricted by short penetration depth and three-dimensional resolution.

1.2.2. Two-Photon Illumination

Two-photon optogenetic excitation facilitates the excitation of subcellular, single or multiple cells in

living brain tissues at unprecedented depth with high spatiotemporal resolution (Prakash *et al.*, 2012; Accanto *et al.*, 2023). The advancement brought by two-photon optogenetics is mainly ascribed to two main factors: (i) the nonlinear absorption process in two-photon excitation allows high spatial resolution in three dimensions, and (ii) the use of infrared wavelength that allows deep light penetration in tissues due to less scattering (Ronzitti *et al.*, 2017).

Two-photon optogenetic excitation of neurons was demonstrated with ChR2 (Rickgauer & Tank, 2009), red-light activated ChR from *Volvox carteri* C1V1 (Packer *et al.*, 2012, 2015; Prakash *et al.*, 2012; Rickgauer *et al.*, 2014), ReaChR (Chaigneau *et al.*, 2015) and Chronos (Ronzitti *et al.*, 2016). Furthermore, recent two-photon holographic photoactivation of ChroME- and CoChR-expressing neurons have reported optogenetic control of spiking with sub-millisecond temporal precision (Ronzitti *et al.*, 2016; Mardinly *et al.*, 2018; Shemesh *et al.*, 2017). Along with excitation, the emergence of anion-conducting ChRs, namely GtACR2, having various advantages over light-driven chloride pumps, has enabled two-photon holographic suppression at 920 nm and stimulus power 30 mW (Forli *et al.*, 2018). Recent efforts for designing opsins with larger conductance have overcome limitations associated with early mutants with poor conductance and light sensitivity. Newly discovered opsins, namely, ChroME2f, ChroME2s, ChRmine, and its mutants, have enabled large network excitation through parallel and scanning approaches (Sridharan *et al.*, 2022; Marshel *et al.*, 2019).

1.2.3. Patterned Illumination

Patterned illumination offers experimentalists the capability to recreate highly particular, quasi-physiological patterns of activity in the intact brain with optogenetics. Light patterning techniques facilitate fast, remote modification of the spatial configuration of light delivered to tissue without the requirement to intervene in the optical setup during the experiment (Emiliani *et al.*, 2022). Such techniques can be beneficial for stimulating a subpopulation of genetically identical neurons or targeting subcellular structures, such as dendrites, axons, or dendritic spines (Papagiakoumou *et al.*, 2013).

Recent studies have used two-photon illumination for changing membrane potential in dendritic spines, which contributes to the most excitatory neurotransmission in the nervous system and must be

critical for brain function (Cornejo *et al.,* 2022). Spatial selectivity of synaptic compartments in the whole neuron via two-photon illumination has enabled probing important phenomena that include synaptic function, synaptic plasticity, dendritic integration, and neurological diseases (Cornejo *et al.,* 2022; Hage *et al.,* 2022).

2. COMPUTATIONAL OPTOGENETICS

Theoretical understanding of the underlying mechanisms of optogenetic switching of neurons offers deeper insights and a fast way to optimize photostimulation conditions for better control. Computational modeling is thus fundamental to understanding how light-induced ionic transport across cell membranes can be tailored for probing cellular physiology and to create new treatments for neurological and psychiatric illnesses. It has the capability to guide experimentalists to design and engineer new tools and methods for different applications in and beyond neuroscience (Nikolic *et al.,* 2009; Foutz *et al.,* 2012; Williams *et al.,* 2013; Evans *et al.,* 2016, Stefanescu *et al.,* 2013). Theoretical models help in rapidly conducting virtual experiments with a wide range of opsins and their expression in different types of cells.

Computational modeling of optogenetic switching of cells needs multiple levels of theoretical analysis, from molecular modeling through kinetic models to even network-level models of opsin-expressing cells. For modeling photocurrent kinetics, transition rate models of the photocycle ChR2 and its mutants were reported earlier (Nikolic *et al.,* 2009). Theoretical analysis has suggested that a slight change in the turn-off, turn-on or recovery time of opsin photocurrent may substantially affect the burst activity pattern of the neurons-expressing these opsins. Recently, theoretical modeling and analysis of photocurrent kinetics of various new opsins have been reported (Saran *et al.,* 2018; Gupta *et al.*, 2019; Bansal *et al.,* 2020a, 2020b, 2021, 2022b, 2023; Pyari *et al.,* 2022, 2023). Integration of these photocurrent models with biophysical model of neurons allows the modeling of optogenetically excited action potentials in the brain, heart, and retina. It has been shown that the simulated results using the formulated theoretical models are in excellent agreement with the reported experimental results, and enable optimization of optogenetic control.

3. CHALLENGES

Low-power optogenetic excitation of opsin-expressing cells is a major challenge, as light illumination

from the surface of the tissue drastically reduces while propagating in biological tissue (Emiliani *et al.*, 2022). Thus, continuous efforts are being made to discover and engineer opsins with higher sensitivity. In *in vivo* excitation, the use of higher irradiances results in unwanted heating and may cause thermal damage in the tissue. Reported experiments have shown that commonly used light powers increase the temperature by 0.2–2° C, which is sufficient for altering ongoing spiking in the network (Senova *et al.,* 2017; Owen *et al.,* 2019). Thus, careful consideration of light delivery parameters is required. Although newly discovered opsins have greater sensitivity than earlier opsins, the light illumination conditions in *in vivo* experiments using these opsins have a wide scope of optimization (Emiliani *et al.,* 2022).

Opsins with specific ion-selectivity have long been desired as their potential applications to treat neurological disorders created from ionic imbalances (Emiliani *et al.,* 2022). It was reported that ion-specific rhodopsins would be invaluable tools for studying downstream physiological responses to specific ions/second messengers both in neuroscience and beyond (McIsaac *et al.,* 2015). Recently, discovered Ca^{2+} and K^{+} selective light-gated ChRs have significantly improved the control over the neuronal activity. However, instantaneous monitoring of change in ionic concentrations due to sustained opening of these channels is very important for their effective use. Existing computational models of optogenetic switching with non-selective ChRs are not capable of doing so. Thus, there is an urgent need for advanced theoretical models for these ion-selective channels to not only better understand their impact but also to explore their applications.

Two-photon excitation is still challenging due to the intrinsic complexity of setup and illumination patterns. Due to the diverse characteristics of light-gated channels that include conductivity, light-sensitivity and photocurrent kinetics, efficient illumination protocols are therefore essential for efficient excitation of cells (Papagiakoumou *et al.,* 2013). A common challenge for two-photon optogenetics is the need to enlarge the excitation surface (Emiliani *et al.,* 2022). Due to a much smaller cross-section, higher photon fluxes are required to generate similar excitation rates (Carrillo-Reid *et al.,* 2019; Marshel *et al.,* 2019). Gain-of-function approaches are powerful. However, investigating the causality between the neural dynamics and functions is also a challenging task in two-photon excitation (Adesnik and Abdeladim, 2021). Furthermore, the effective spatial resolution of

two-photon excitation is highly power-dependent. If the laser power on a target neuron exceeds the power range where the photocurrent has a quadratic dependence on excitation power, the axial resolution will deteriorate, whether using focused or parallel illumination. In such cases, there could be a substantial increase in the likelihood of off-target activation (Adesnik and Abdeladim, 2021).

The theoretical framework of multi-compartmental optogenetic excitation of pyramidal neurons expressed with ChR2 was reported by integrating the photoconductivity of ChR2 using a six-state model into NEURON simulation environment for better representation (Hines & Carnevale, 1997). The model allows studying the dynamics of optogenetic perturbations of three-dimensional morphologically realistic neurons and neuronal circuits (Grossman *et al.,* 2013). Although recently reported studies on compartment-specific optogenetic excitation of neurons have provided novel insights into signal processing and synaptic plasticity, experimental optimization of the light delivery conditions in such a complex system is a very challenging task. Thus, theoretical models of recently reported somatic, dendritic, or axonic optogenetic stimulations with newly discovered opsins are urgently needed.

4. MOTIVATION

Computational modeling of optogenetic-excitation of cells has made significant contributions in the experimentally driven field of optogenetics. Although very few theoretical models of photocurrent in earlier opsins and optogenetic excitation of neurons by considering neuron as a single compartment have been reported in this area, these theoretical studies have resulted in various new insights and contributed to our knowledge. The newly discovered opsins have the potential to overcome existing challenges in the field, but there are no theoretical models of these opsins reported to date. Also, there is a wide scope of optimization in two-photon and compartment-specific optogenetic excitation. However, no accurate theoretical model has been reported as yet. Thus, we are motivated to carry out a detailed computational study of new opsins-expressing cells, and the activation under two-photon and compartment-specific excitation. It will allow us to not only better understand these complex experiments but also help in optimizing and guiding future experiments.

5. OBJECTIVES

The objective of the proposed thesis is to conduct a comprehensive theoretical study of optical

switching of neurons expressed with light-gated ion-channels to achieve fast, low-power, and high-fidelity control over electrical signals inside the brain. In optogenetics, intense efforts are continuously being made to design new opsins with greater spectral sensitivity, conductance, molecular specificity, binding affinity and kinetics.

Recently, ion-specific ChRs have been discovered and engineered for the effective control of cells. Calcium has a widespread role in cellular signaling, plasticity, muscle contraction, apoptosis and cell growth processes, and metabolic excitation (Berridge *et al.,* 2003, Chindemi *et al.,* 2022). However, probing these phenomena has been limited due to the poor selection of calcium ions over other ions in light-gated channels. Recently, a genetic mutation in ChRs has led to Ca^{2+} selective ChR, namely CapChR1, CapChR2 and C2-LC (Lahore *et al.,* 2022). These Ca^{2+} selective channels have broad applicability in the reversible and spatiotemporally precise control of Ca^{2+} dynamics and other relevant processes. Furthermore, K^+ selective ChRs, namely WiChR, HcKCR1 and HcKCR2 have also been discovered. Higher selectivity for K^+ ions over Na^+ of these light-gated channels is promising for the study and potential treatment of potassium channelopathies such as epilepsy, Parkinson's disease and long-QT syndrome, and other cardiac arrhythmias (Govorunova *et al.,* 2022). A recent study has shown that WiChR is well expressed in neurons and cardiac myocytes and showed reduced depolarization at negative membrane potentials and prolonged suppression of electrical activity (Vierock *et al.*, 2022).

Recently discovered pump-like ChR, named ChRmine, exhibits extremely high-sensitivity and large conductance, which allows ultra-low power, minimally invasive optogenetic control of cells in the brain and the heart (Marshel *et al.*, 2019; Chen *et al.,* 2021). More recently, two new promising mutants of ChRmine, named hsChRmine (accelerated-kinetics variant (H33R)) and rsChRmine (red-shifted variant (I146M/G174S)) with altered kinetics, light-sensitivity, and conductance have been discovered. These new mutants have promising features for designing high-frequency low-power optogenetics control under wide-field as well as two-photon excitation (Kishi *et al.,* 2022). These newly discovered opsins have the potential to overcome existing challenges in optogenetics and have a wide scope of applications. However, no theoretical study of these newly discovered light-gated channels has been reported as yet. It is proposed to formulate accurate theoretical models of these

newly discovered opsins and determine optimal conditions for low-power, high-fidelity, high-frequency, sustained, and deep optogenetic switching of neurons.

The deep and spatially precise control of neuronal activity within the network is essential for understanding the role of an individual cell in the behavioral outcome. Although advanced genetic expression methods allow locating the opsin into specific neurons or subcellular regions, visible light used in wide-field illumination of cells cannot penetrate deeper into the biological tissue due to high absorption and scattering. Although two-photon excitation with highly intense laser spot can overcome the limitations, it is limited due to poor sensitivity and low conductance of existing opsins. Recently, new opsins, namely ChroME2f, ChroME2s, ChRmine, hsChRmine, and rsChRmine, with high-sensitivity and large conductance have been discovered to meet the challenges. It is proposed to formulate an accurate theoretical model of two-photon optical switching of neurons with newly discovered opsins and conduct a detailed theoretical analysis to achieve low-power, high-frequency, and high-fidelity control.

Recently reported compartment-specific optogenetic stimulations have provided useful insights into signal processing under different conditions. It is shown that the effective current resulting from light-gated channels varies dramatically due to local ion concentration gradient differences at the somatic, dendritic, and axonic locations. For example, ACRs are very useful for shunting inhibition. However, they can exert excitatory effects in axons and presynaptic terminals due to higher intracellular chloride concentration (Wiegert *et al.*, 2017). Thus, computational models of compartment-specific optogenetic excitation are urgently needed to understand the direction of signal propagation and design optimized patterns for the desired action.

The specific objectives of the study are outlined as follows:

(i) It is proposed to formulate accurate theoretical model of optical switching of neurons expressed with newly discovered channelrhodopsins (CapChR2, rsChRmine, hsChRmine, and WiChR).

(ii) It is proposed to optimize photostimulation and physiological conditions that include light intensity, pulse width, pulse timing, pulse frequency and expression level for low-power high-fidelity control.

(iii) It is proposed to formulate accurate theoretical model of two-photon optical excitation of

neurons expressed with newly discovered opsins (ChRmine and its mutants) and compare their response with wide field illumination.

(iv) It is proposed to formulate accurate biophysical multi-compartmental model of optical excitation of neurons to study the effect of spatial distribution of light for probing into the neural spiking.

6. METHODOLOGY

The objective wise methodology for doing the research work is described in the following steps:

(i) Formulation of accurate theoretical model of optogenetic switching of neurons with newly discovered opsins and validation of the model with reported experimental results ((Lahore *et al.,* 2022, Kishi *et al.* 2022, Govorunova *et al.* 2022)

(ii) Detailed study of the effect of different photostimulation and physiological factors to determine optimal conditions to address the existing challenges.

(iii) These kinetic models will then be integrated with accurate biophysical model of hippocampal neurons and fast-spiking interneurons using Hodgkin-Huxley and Wang-Buzaski neuron models, respectively.

(iv) The results of neuronal spike control, reported in recent path-breaking experimental studies (Lahore *et al.,* 2022; Kishi *et al.,* 2022) will be simulated using the formulated integrated biophysical model. Consequently, optimal conditions for photostimulation that include light intensity, pulse-width, wavelength and frequency will be determined.

(v) The multi-compartmental model of optical excitation of neurons will be formulated for probing into neuronal signaling (Grossman *et al.,* 2013).

(vi) An accurate two-photon optogenetic stimulation model will be formulated and the optimal conditions to overcome existing challenges will be determined.

7. EXPECTED OUTCOME

The proposed research work will be an important research contribution in the field of optogenetics. It will result in accurate theoretical models of optical excitation of cells expressed with newly discovered opsins. It will also provide deeper understanding of the mechanism behind generation of photocurrent through these channels and light-evoked spiking in targeted cells. The proposed research encompasses

optical switching of opsin-expressing cells under wide field and two-photon illumination, and biophysically detailed multi-compartmental models of optogenetic excitation. It will provide optimal values of various physical parameters that include intensity, pulse-width, wavelength and frequency, which would be helpful in designing efficient experiments. It would also lead to designs of efficient optogenetic based neuroprosthetic devices.

8. WORK ALREADY DONE

An extensive literature survey has been carried out in the field of optogenetics. Theoretical results reported in previous research papers have been reproduced. Theoretical model of the photocurrent of newly discovered opsins, named CapChR2 has been formulated and validated by comparing results with reported experimental results. Theoretical analysis of CapChR2 has been done which includes variation of irradiance, pulse-width, peak photocurrents and plateau photocurrents. Broad literature review of synaptic plasticity has been carried out and an existing mathematical model has been found in order to find out new results.

References

Accanto N., Blot F. G., Lorca-Camara A., Zampini V., Bui F., Tourain C., Badt N., Katz O., Emiliani V., "A flexible two-photon fiberscope for fast activity imaging and precise optogenetic photostimulation of neurons in freely moving mice," *Neuron*, ***111***(2), 176-189, (2023).

Adamczyk A. K., Zawadzki P., "The memory-modifying potential of optogenetics and the need for neuroethics," *Nanoethics*, ***14***(3), 207-225, (2020).

Adesnik H., Abdeladim L., "Probing neural codes with two-photon holographic optogenetics," *Nat. Neurosci.*, ***24***(10), 1356-1366, (2021).

Bansal A., Shikha S., Zhang Y., "Towards translational optogenetics." *Nat. Biomed. Eng.*, ***7***(4), 349-369, (2023).

Bansal H., Gupta N., Roy S., "Comparison of low-power, high-frequency and temporally precise optogenetic inhibition of spiking in NpHR, eNpHR3.0 and Jaws-expressing neurons," *Biomed. Phys. Eng. Exp.*, ***6***(4), 1-34, (2020a).

Bansal H., Gupta N., Roy S., "Theoretical analysis of low-power bidirectional optogenetic control of high-frequency neural codes with single spike resolution," *Neuroscience*, ***449***, 165-188,

(2020b).

Bansal H., Gupta N., Roy S., “Theoretical analysis of optogenetic spiking with ChRmine, bReaChES and CsChrimson-expressing neurons for retinal prostheses,” *J. Neural Eng.*, ***18***(4), (2021).

Bansal H., Pyari G., Roy S., “Co-expressing fast channelrhodopsins with step-function opsin overcomes spike failure due to photocurrent desensitization in optogenetics: A theoretical study,” *J. Neural Eng.*, ***19***(2), 026032, (2022b).

Bansal H., Pyari G., Roy S., “Optogenetic Generation of Neural Firing Patterns with Temporal Shaping of Light Pulses.” *Photonics*, ***10***(5), 571 (2023).

Bedbrook C. N., Yang K. K., Robinson J. E., Mackey E. D., Gradinaru V., Arnold F. H., “Machine learning-guided channelrhodopsin engineering enables minimally invasive optogenetics.” *Nat. Methods*, ***16***(11), 1176-1184, (2019).

Berndt A., Yizhar O., Gunaydin L. A., Hegemann P., Deisseroth K., “Bi-stable neural state switches,” *Nat. Neurosci.*, ***12***(2), 229-234, (2009).

Berridge M. J., Bootman, M. D., Roderick, H. L., “Calcium signalling: dynamics, homeostasis and remodeling,” *Nat. Rev. Mol. Cell Biol.*, ***4***, 517–529, (2003).

Boyden E. S., Zhang F., Bamberg E., Nagel G., Deisseroth K., “Millisecond-timescale, genetically targeted optical control of neural activity,” *Nat. Neurosci.*, ***8***(9), 1263-1268, (2005).

Carrillo-Reid L., Han S., Yang W., Akrouh A., Yuste R., “Controlling visually guided behavior by holographic recalling of cortical ensembles,” *Cell*, ***178***(2), 447-457, (2019).

Cehajic-Kapetanovic J., Singh M. S., Zrenner E., MacLaren, R. E., “Bioengineering strategies for restoring vision.” *Nat. Biomed. Eng.*, ***7***(4), 387-404, (2023)..

Chaigneau E., Ronzitti E., Gajowa M. A., Soler-Llavina G. J., Tanese D., Brureau A. Y., Papagiakoumou E., Zeng H. Emiliani V., “Two-photon holographic stimulation of ReaChR,” *Front. Cell. Neurosci.*, ***10***, 234, (2016).

Chen R., Gore F., Nguyen Q. A., Ramakrishnan C., Patel S., Kim S. H., Raffiee M., KimY. S., Hsueh B., Krook-Magnusson E., Soltesz I., Deisseroth K., “Deep brain optogenetics without intracranial surgery,” *Nat. Biotechnol.*, ***39***(2), 161-164, (2021).

Chindemi G., Abdellah M., Amsalem O., Benavides-Piccione R., Delattre V., Doron M., Ecker A.,

Jaquier A. T., King J., Pramod K., Monney C., Perin R., Rossert C., Tuncel A. M., Geit W.V., Javier D., Graupner M., Segev I., Markram H., Muller E. B. “A calcium-based plasticity model for predicting long-term potentiation and depression in the neocortex.” *Nat. Commun.*, ***13***(1), 3038, (2022).

Cho Y. U., Lim S. L., Hong J. H., Yu K. J., “Transparent neural implantable devices: A comprehensive review of challenges and progress.” *npj Flexible Electron.*, ***6***(1), 53, (2022).

Christie J. M., Zurbriggen M. D., “Optogenetics in plants,” *New Phytol.*, ***229***(6), 3108-3115, (2021).

Chuong A. S., Miri M. L., Busskamp V., Matthews G. A., Acker L. C., Sorensen A. T., Young A., Klapoetke N. C., Henninger M. A., Kodandaramaiah S. B., Ogawa M., Ramanlal S. B., Bandler R. C., Allen B. D., Forest C. R., Chow B. Y., Han X., Lin Y., Tye K. M., Roska B., Cardin J. A., Boyden E. S., “Noninvasive optical inhibition with a red-shifted microbial rhodopsin,” *Nat. Neurosci.*, ***17***(8), 1123-1129, (2014).

Cohen E. M., Knapman F. L., Bilston, L. E., “Optogenetic control of muscles: Potential uses and limitations.” *Hum. Gene Ther*, ***34***(9-10), 416-429, (2023).

Cornejo V. H., Ofer N., Yuste R., “Voltage compartmentalization in dendritic spines in vivo,” *Science*, ***375***(6576), 82-86, (2022).

Cury R. G., Pavese N., Aziz T. Z., Krauss J. K., Moro E., the Neuromodulation of Gait Study Group from Movement Disorders Society., “Gaps and roadmap of novel neuromodulation targets for treatment of gait in Parkinson’s disease,” *npj Parkinson's Dis.*, ***8***(1), 8, (2022).

Deisseroth K., “Optogenetics: 10 years of microbial opsins in neuroscience,” *Nat. Neurosci.*, ***18***(9), 1213-1225, (2015).

Emiliani V., Entcheva E., Hedrich R., Hegemann P., Konrad K. R., Luscher C., Mahn M., Pan Z., Sims R., Vierock J., “Optogenetics for light control of biological systems,” *Nat. Rev. Methods Primers.*, ***2***(1), 55, (2022).

Engelhard C., Chizhov I., Siebert F., Engelhard M., “Microbial halorhodopsins: light-driven chloride pumps,” *Chem. Rev.*, ***118***(21), 10629-10645, (2018).

Entcheva E., Kay M. W., “Cardiac optogenetics: a decade of enlightenment,” *Nat. Rev. Cardiol.*, ***18***(5), 349-367, (2021).

Evans B. D., Jarvis S., Schultz S. R., Nikolic K., "PyRhO: a multiscale optogenetics simulation platform," *Front. Neuroinform*, ***10***, 8, (2016).

Forli A., Vecchia D., Binini N., Succol F., Bovetti S., Moretti C., Nespoli F., Mahn M., Baker C. A., Bolton M. M., Yizhar O., "Two-photon bidirectional control and imaging of neuronal excitability with high spatial resolution in vivo," *Cell Rep.*, ***22***(11), 3087-3098, (2018).

Foutz T. J., Arlow R. L., McIntyre C. C., "Theoretical principles underlying optical stimulation of a Channelrhodpsin-2 positive pyramidal neuron," *J. Neurophysiol.*, ***107***(12), 3235-3245, (2012).

Gao T. T., Oh T. J., Mehta K., Huang Y. A., Camp T., Fan H., Zhang K., "The clinical potential of optogenetic interrogation of pathogenesis," *Clin. Trans. Med.*, ***13***(5), e1243, (2023).

Geng Y., Li Z., Zhu J., Du C., Yuan F., Cai X., Ali A., Yang J., Tang C., Cong Z., Ma C., "Advances in Optogenetics Applications for Central Nervous System Injuries," *J. Neurotrauma,* (2023). https//:doi.10.1089/neu.2022.0290.

Gong X., Mendoza-Halliday D., Ting J. T., Kaiser T., Sun X., Bastos A. M., Wimmer R.D., Guo B., Chen Q., Zhou Y., Pruner M., Wu C. W. H., Park D., Deisseroth K., Barak B., Boyden E. S., Miller E. K., Halassa M. M., Fu Z., Bi G., Feng G., "An ultra-sensitive step-function opsin for minimally invasive optogenetic stimulation in mice and macaques," *Neuron*, ***107***(1), 38-51, (2020).

Govorunova E. G., Gou Y., Sineshchekov O. A., Li H., Lu X., Wang Y., Brown L.S., St-Pierre F., Xue M., Spudich J. L., "Kalium ChRs are natural light-gated potassium channels that mediate optogenetic inhibition," *Nat. Neurosci.*, ***25***(7), 967-974, (2022).

Govorunova E. G., Sineshchekov O. A., Janz R., Liu X., Spudich J. L., "Natural light-gated anion channels: A family of microbial rhodopsins for advanced optogenetics," *Science*, ***349***(6248), 647-650, (2015).

Govorunova E. G., Sineshchekov O. A., Li H., Spudich J. L., "Diversity and molecular mechanisms of ChRs," *Biophys. J.*, ***122***(3), 18a, (2023).

Govorunova E. G., Sineshchekov O. A., Li H., Wang Y., Brown L. S., Spudich J. L., "RubyACRs, nonalgal anion channelrhodopsins with highly red-shifted absorption," *Proc. Natl. Acad. Sci. U.S.A.*, ***117***(37), 22833-22840, (2020).

Gradinaru V., Zhang F., Ramakrishnan C., Mattis J., Prakash R., Diester I., Goshen I., Thompson K. R., Deisseroth K., "Molecular and cellular approaches for diversifying and extending optogenetics," *Cell*, ***141***(1), 154-165, (2010).

Grossman N., Simiaki V., Martinet C., Toumazou C., Schultz S. R., Nikolic K., "The spatial pattern of light determines the kinetics and modulates backpropagation of optogenetic action potentials." *J. Comput. Neurosci.*, ***34***, 477-488, (2013).

Gunaydin L. A., Yizhar O., Berndt A., Sohal V. S., Deisseroth K., Hegemann P., "Ultrafast optogenetic control," *Nat. Neurosci.*, *13*(3), 387-392, (2010).

Gupta N., Bansal H., Roy S., "Theoretical optimization of high-frequency optogenetic spiking of red-shifted very fast-Chrimson-expressing neurons," *Neurophoton.*, ***6***(2), 025002, (2019).

Guru A., Post R. J., Ho Y. Y., Warden M. R., "Making sense of optogenetics," *Int. J. Neuropsychopharmacol.*, ***18***(11), pyv079, (2015).

Hage T. A., Bosma-Moody A., Baker C. A., Kratz M. B., Campagnola L., Jarsky T., Zeng H., Murphy G. J., "Synaptic connectivity to L2/3 of primary visual cortex measured by two photon optogenetic stimulation," *eLife*, ***11***, e71103, (2022).

Han X., Boyden E. S., "Multiple-color optical activation, silencing, and desynchronization of neural activity, with single-spike temporal resolution," *PLoS One*, ***2***(3), 299, (2007).

Hines M. L., Carnevale N. T., "The NEURON simulation environment." *Neural Comput.*, ***9***(6), 1179-1209, (1997).

Joshi J., Rubart M., Zhu W., "Optogenetics: background, methodological advances and potential applications for cardiovascular research and medicine," *Front. Bioeng. Biotechnol.*, ***7***, 466, (2020).

Kim C. K., Yang S. J., Pichamoorthy N., Young N. P., Kauvar I., Jennings J. H., Lerner T. N., Berndt A., Lee S. Y., Ramakrishnan C., Davidson T. J., Inoue M., Bito H., Deisseroth, K., "Simultaneous fast measurement of circuit dynamics at multiple sites across the mammalian brain." *Nat. Methods*, ***13***(4), 325-328, (2016).

Kim C. K., Adhikari A., Deisseroth K., "Integration of optogenetics with complementary methodologies in systems neuroscience," *Nat. Rev. Neurosci.*, ***18***(4), 222-235, (2017).

Kishi K. E., Kato H. E., “Pump-like Channelrhodopsins: Not just bridging the gap between ion pumps and ion channels,” *Curr. Opin. Struct. Biol.*, ***79***, 102562, (2023).

Kishi K. E., Kim Y. S., Fukuda M., Inoue M., Kusakizako T., Wang P. Y., Ramakrishnan C., Byrne E. F. X., Elina T., Paggi J. M., Matsui T. E., Yamashita K., Nagata T., Masae K., Quirin S., Lo M., Benster T., Uemura T., Liu K., Shibata M., Kato H. E., “Structural basis for channel conduction in the pump-like channelrhodopsin ChRmine,” *Cell*, ***185***(4), 672-689, (2022).

Klapoetke N. C., Murata Y., Kim S. S., Pulver S. R., Birdsey-Benson A., Cho Y. K., Morimoto Y. K., Chuong A.S., Carpenter E.J., Tian Z., Wang J., Xie Y., Yan Z., Zhang Y., Chow B.Y., Surek B., Melkonian M., Jayaraman V., Paton M. C., Wong G. K. S., Boyden E. S., “Independent optical excitation of distinct neural populations,” *Nat. Methods*, ***11***(3), 338-346, (2014).

Kojima K., Kawanishi S., Nishimura Y., Hasegawa M., Nakao S., Nagata Y., Sudo Y., “A blue-shifted anion channelrhodopsin from the Colpodellida alga *Vitrella brassicaformis*,” *Sci. Rep.*, ***13***(1), 6974, (2023).

Krohn S., von Schwanenflug N., Waschke L., Romanello A., Gell M., Garrett D. D., Finke C., “A spatiotemporal complexity architecture of human brain activity,” *Sci. Adv.*, ***9***(5), eabq3851, (2023).

Krueger D., Izquierdo E., Viswanathan R., Hartmann J., Pallares Cartes C., De Renzis S., “Principles and applications of optogenetics in developmental biology,” *Development*, ***146***(20), dev175067, (2019).

Lahore R. G. F., Pampaloni N. P., Peter E., Heim M. M., Tillert L., Vierock J., Oppermann J., Walther J., Schmitz D., Owald D., Plested A. J. R., Rost B. R., Hegemann P., “Calcium-permeable channelrhodopsins for the photocontrol of calcium signaling,” *Nat. Commun.*, ***13***(1), 7844, (2022).

Li H., Wang J., Fang Y., “Recent developments in multifunctional neural probes for simultaneous neural recording and modulation,” *Microsyst. Nanoeng.*, ***9***(1), 4, (2023a).

Li L., Lu L., Ren Y., Tang G., Zhao Y., Cai X., Shi Z., Ding H., Liu C., Cheng D., Xie Y., Wang H., Fu X., Yin L., Luo M., Sheng X., “Colocalized, bidirectional optogenetic modulations in freely behaving mice with a wireless dual-color optoelectronic probe,” *Nat. Commun.*, ***13***(1),

839, (2022).

Li Z., Zheng Y., Diao X., Li R., Sun N., Xu Y., Si K., "Robust and adjustable dynamic scattering compensation for high-precision deep tissue optogenetics," *Commun. Biol.*, ***6***(1), 128, (2023b).

Lin A., Witvliet D., Hernandez-Nunez L., Linderman S. W., Samuel A. D., Venkatachalam V., "Imaging whole-brain activity to understand behavior," *Nat. Rev. Phys.*, ***4***(5), 292-305, (2022).

Lin J. Y., Knutsen P. M., Muller A., Kleinfeld D., Tsien R. Y., "ReaChR: a red-shifted variant of channelrhodopsin enables deep transcranial optogenetic excitation," *Nat. Neurosci.*, ***16***(10), 1499-1508, (2013).

Lin Y., Yao Y., Zhang W., Fang Q., Zhang L., Zhang Y., Xu Y., "Applications of upconversion nanoparticles in cellular optogenetics," *Acta Biomater.*, ***135***, 1-12, (2021).

Lindner F., Diepold A., "Optogenetics in bacteria–applications and opportunities," *FEMS Microbiol. Rev.*, ***46***(2), fuab055, (2022).

Liu C., Xin Q., Qin C., Jiang M., Lo G. V., Dou Y., Yuan S. "The Mechanism of Channel Opening of Anion Channelrhodopsin GtACR1 : A Molecular Dynamics Simulation," *Processes*, ***11***(2), 510, (2023).

Mager T., Lopez de la Morena D., Senn V., Schlotte J., D Errico A., Feldbauer K., Wrobel C., Jung S., Bodensiek K., Rankovic V., Browne L., "High frequency neural spiking and auditory signaling by ultrafast red-shifted optogenetics," *Nat. Commun.*, ***9***(1), 1750, (2018) .

Mardinly A. R., Oldenburg I. A., Pegard N. C., Sridharan S., Lyall E. H., Chesnov K., Brohawn S. G., Waller L., Adesnik H., "Precise multimodal optical control of neural ensemble activity," *Nat. Neurosci.*, ***21***(6), 881-893, (2018).

Marin M. D. C., Konno M., Yawo H., Inoue K., "Converting a Natural-Light-Driven Outward Proton Pump Rhodopsin into an Artificial Inward Proton Pump," *J. Am. Chem. Soc.*, (2023). https://doi.org/10/1021/jacs.2c12602

Marshel J. H., Kim Y. S., Machado T. A., Quirin S., Benson B., Kadmon J., Raja C., Chibukhchyan A., Ramakrishnan C., Inoue M., Shane J. C., McKnight D. J., Yoshizawa S., Kato H. E.,

Ganguli S., Deisseroth, K., “Cortical layer–specific critical dynamics triggering perception,” *Science*, ***365***(6453), 1-22, (2019).

McIsaac T. L., Lamberg E. M., Muratori L. M., “Building a framework for dual task taxonomy,” *Biomed Res. Int.*, 1-10, (2015). https://doi.org/10.1155/2015/591475

Nagel G., Szellas T., Huhn W., Kateriya S., Adeishvili N., Berthold P., Ollig D., Hegemann P., Bamberg, E., “Channelrhodopsin-2, a directly light-gated cation-selective membrane channel.” *Proc. Natl. Acad. Sci. U.S.A.*, ***100***(24), 13940-13945, (2003).

Nikolic K., Grossman N., Grubb M. S., Burrone J., Toumazou C., Degenaar P., “Photocycles of Channelrhodopsin-2,” *Photochem. Photobiol.*, ***85***(1), 400-411, (2009).

Owen S. F., Liu M. H., Kreitzer A. C., “Thermal constraints on in vivo optogenetic manipulations,” *Nat. Neurosci.*, ***22***(7), 1061-1065, (2019).

Packer A. M., Peterka D. S., Hirtz J. J., Prakash R., Deisseroth K., Yuste R., “Two-photon optogenetics of dendritic spines and neural circuits,” *Nat. Methods*, ***9***(12), 1202-1205, (2012).

Packer A. M., Russell L. E., Dalgleish H. W., Häusser M., “Simultaneous all-optical manipulation and recording of neural circuit activity with cellular resolution in vivo,” *Nat. Methods*, ***12***(2), 140-146, (2015).

Panzeri S., Moroni M., Safaai H., Harvey C. D., “The structures and functions of correlations in neural population codes,” *Nat. Rev. Neurosci.*, ***23***(9), 551-567, (2022).

Papagiakoumou E., Begue A., Leshem B., Schwartz O., Stell B. M., Bradley J., Oron D., Emiliani, V., “Functional patterned multiphoton excitation deep inside scattering tissue,” *Nat. Photoni.*, ***7***(4), 274-278, (2013).

Parusel S., Yi M. H., Hunt C. L., Wu L. J., “Chemogenetic and optogenetic manipulations of microglia in chronic pain,” *Neurosci. Bull.*, ***39***(3), 368-378, (2023).

Prakash R., Yizhar O., Grewe B., Ramakrishnan C., Wang N., Goshen I., Packer A. M., Peterka D. S., Yuste R., Schnitzer M. J., Karl D., “Two-photon optogenetic toolbox for fast inhibition, excitation and bistable modulation.” *Nat. Methods*, ***9***(12), 1171-1179, (2012).

Pyari G., Bansal H., Roy S., “Ultra-low power deep sustained optogenetic excitation of human ventricular cardiomyocytes with red-shifted opsins: a computational study,” *J. Physiol.*,

600(21), 4653-4676, (2022).

Pyari G., Bansal H., Roy S., "Optogenetically mediated large volume suppression and synchronized excitation of human ventricular cardiomyocytes." *Pflug. Arch. Eur. J. Physiol.*, 1-25, (2023). https://doi.org/10.1007/s00424-023-02831-x

Repina N. A., Rosenbloom A., Mukherjee A., Schaffer D. V., Kane R. S., "At light speed: advances in optogenetic systems for regulating cell signaling and behavior," *Annu. Rev. Chem. Biomol. Eng.*, ***8***, 13-39, (2017).

Rickgauer J. P., Deisseroth K., Tank D. W., "Simultaneous cellular-resolution optical perturbation and imaging of place cell firing fields," *Nat. Neurosci.*, ***17***(12), 1816-1824, (2014).

Rickgauer J. P., Tank D. W., "Two-photon excitation of Channelrhodopsin-2 at saturation," *Proc. Natl. Acad. Sci. U.S.A.*, ***106***(35), 15025-15030, (2009).

Ronzitti E., Ventalon C., Canepari M., Forget B. C., Papagiakoumou E., Emiliani V., "Recent advances in patterned photostimulation for optogenetics," *J. Opt.*, ***19***(11), 113001, (2017).

Sahel J. A., Boulanger-Scemama E., Pagot C., Arleo A., Galluppi F., Martel J. N., Esposti S.D., Delaux A., Aubert J. B. S., Montleau C., Gutman E., Audo I., Duebel J., Picaud S., Dalkara D., Blouin L., Taiel M., Roska B., "Partial recovery of visual function in a blind patient after optogenetic therapy," *Nat. Med.*, ***27***(7), 1223-1229, (2021).

Saran S., Gupta N., Roy S., "Theoretical analysis of low-power fast optogenetic control of firing of Chronos-expressing neurons," *Neurophoton.*, ***5***(2), 025009, (2018).

Scangos K. W., State M. W., Miller A. H., Baker J. T., Williams L. M., "New and emerging approaches to treat psychiatric disorders," *Nat. Med.*, ***29***, 317–333 (2023).

Senova S., Scisniak I., Chiang C. C., Doignon I., Palfi S., Chaillet A., Martin C., Pain F., "Experimental assessment of the safety and potential efficacy of high irradiance photostimulation of brain tissues," *Sci. Rep.*, ***7***(1), 43997, (2017).

Seth A. K., Bayne T., "Theories of consciousness," *Nat. Rev. Neurosci.*, ***23***(7), 439-452, (2022).

Shah H. A., Mehta N. H., Saleem M. I., D'Amico R. S., "Connecting the Connectome: A Bibliometric Investigation of the 50 Most Cited Articles," *Clin. Neurol. Neurosurg.*, (2022). https://doi.org/10.1016/j.clineuro.2022.107481

Shemesh O. A., Tanese D., Zampini V., Linghu C., Piatkevich K., Ronzitti E., Papagiakoumou E., Boyden E. S, Emiliani V., "Temporally precise single-cell-resolution Optogenetics," *Nat. Neurosci.*, ***20***(12), 1796-1806, (2017).

Simunovic M. P., Shen W., Lin J. Y., Protti D. A., Lisowski L., Gillies M. C., "Optogenetic approaches to vision restoration," *Exp. Eye Res.*, ***178***, 15-26, (2019).

Sineshchekov O. A., Govorunova E. G., Li H., Wang Y., Melkonian M., Wong G. K. S., Brown L. S., Spudich J. L. (2020). "Conductance mechanisms of rapidly desensitizing cation channelrhodopsins from cryptophyte algae." *Mbio*, ***11***(2), 10-1128.

Sridharan S., Gajowa M. A., Ogando M. B., Jagadisan U. K., Abdeladim L., Sadahiro M., Bounds H. A., Hendricks W. D., Turney T. S., Tayler I., Gopakumar K., "High-performance microbial opsins for spatially and temporally precise perturbations of large neuronal networks," *Neuron*, ***110***(7), 1139-1155, (2022).

Stefanescu R. A., Shivakeshavan R. G., Khargonekar P. P., Talathi S. S., "Computational modeling of ChR2 photocurrent characteristics in relation to neural signaling," *Bull. Math. Biol.*, ***75***, 2208-2240, (2013).

Sung Y. L., Wang T. W., Lin T. T., Lin S. F., "Optogenetics in cardiology: methodology and future applications," *Int J. Arrhythm*, ***23***(1), 1-12, (2022).

Tang X., Shen H., Zhao S., Li N., Liu J., "Flexible brain–computer interfaces," *Nat. Electron.*, ***6***, 109-118, (2023).

Tiwari P., Tolwinski N. S., "Using Optogenetics to Model Cellular Effects of Alzheimer's Disease," *Int. J. Mol. Sci.*, ***24***(5), 4300, (2023).

Urai A. E., Doiron B., Leifer A. M., Churchland A. K., "Large-scale neural recordings call for new insights to link brain and behavior," *Nat. Neurosci.*, ***25***(1), 11-19, (2022).

Vierock J., Peter E., Grimm C., Rozenberg A., Chen I. W., Tillert L., Scalise A. G. C., Casini M., Augustin S., Tanese D., Forget B. C., Peyronnet R., Schneider-Warme F., Emiliani V., Beja O., Hegemann P., "WiChR, a highly potassium-selective ChR for low-light one-and two-photon inhibition of excitable cells," *Sci. Adv.*, ***8***(49), 1-18, (2022).

Vierock J., Rodriguez-Rozada S., Dieter A., Pieper F., Sims R., Tenedini F., Bergs A. C. F.,

Bendifallah I., Zhou F., Zeitzschel N., Ahlbeck J., Augustin S., Sauter K., Papagiakoumou E., Gottschalk A., Soba P., Emiliani V., Engel A. K., Hegemann P., Wiegert, J. S., "BiPOLES is an optogenetic tool developed for bidirectional dual-color control of neurons," *Nat. Commun.*, ***12***(1), 4527, (2021).

Wang Y., Liu S., Wang H., Zhao Y., Zhang X. D., "Neuron devices: emerging prospects in neural interfaces and recognition," *Microsyst. Nanoeng.*, ***8***(1), 128, (2022).

Wang Y., Pan Y., Li H., "What is brain health and why is it important?" *BMJ-BRIT MED J*, ***371***, (2020). https://doi.org/10.1136/bmj.m3683

Wiegert J. S., Mahn M., Prigge M., Printz Y., Yizhar O., "Silencing neurons: tools, applications, and experimental constraints," *Neuron*, ***95***(3), 504-529, (2017).

Williams J. C., Xu J., Lu Z., Klimas A., Chen X., Ambrosi C. M., Cohen I. S., Entcheva E., "Computational optogenetics: empirically-derived voltage-and light-sensitive channelrhodopsin-2 model," *PLoS Comput. Biol.*, ***9***(9), 1003220, (2013).

Xin Q., Zhang W., Yuan S., "The Mechanism of the Channel Opening in channelrhodopsin-2: A Molecular Dynamics Simulation," *Int. J. Mol. Sci.*, ***24***(6), 5667, (2023).

Yang F., Kim S. J., Wu X., Cui H., Hahn S. K., Hong G., "Principles and applications of sono-optogenetics," *Adv. Drug Delivery Rev.*, ***194***, 114711, (2023).

Yang Z., Zeng X., Zhao Y., Chen R., "AlphaFold2 and its applications in the fields of biology and medicine," *Signal Transduct. Target. Ther.*, ***8***(1), 115, (2023).

Yu H., Song L., Duan X., Zhu D., Li N., Pan R., Xu R., Yu X., Ye F., Jiang X., Ye H., Pan Z., Wei S., Jiang Z., "Optogenetics in Taste Research: A Decade of Enlightenment," *Oral Dis.*, (2023). https://doi.org/10.1111/odi.14498

Zaver S. A., Johnson C. J., Berndt A., Simpson C. L., "Live Imaging with Genetically Encoded Physiologic Sensors and Optogenetic Tools," *J. Invest. Dermatol.*, ***143***(3), 353-361, (2023).

Zhang F., Aravanis A. M., Adamantidis A., de Lecea L., Deisseroth K., "Circuit-breakers: optical technologies for probing neural signals and systems," *Nat. Rev. Neurosci.*, ***8***(8), 577-581, (2007).

Zhang F., Prigge M., Beyriere F., Tsunoda S.P., Mattis J., Yizhar O., Hegemann P., Deisseroth K.,

"Red-shifted optogenetic excitation: a tool for fast neural control derived from *Volvox carteri*," *Nat. Neurosci.*, ***11***(6), 631-633, (2008).